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(54) Abstract Title
Method of producing tissue structures

(57) A method of producing a biomedical or biocompatible "scaffold" or tissue structure comprises electrostatically depositing particles of one or more biocompatible materials onto a suitable substrate where the particles interlink. The particles may be mixed with bioactive agents, and cells, cell organelles or micro-organisms may be applied to the scaffold and their growth promoted. One or more implants may be attached to the scaffold or cells. The structures may be used in tissue engineering including tissue repair and cell transplantation. Also disclosed is a method of promoting cell growth by depositing desiccated cells onto a substrate followed by rehydration of the cells.

Electroprocessed
Collagen

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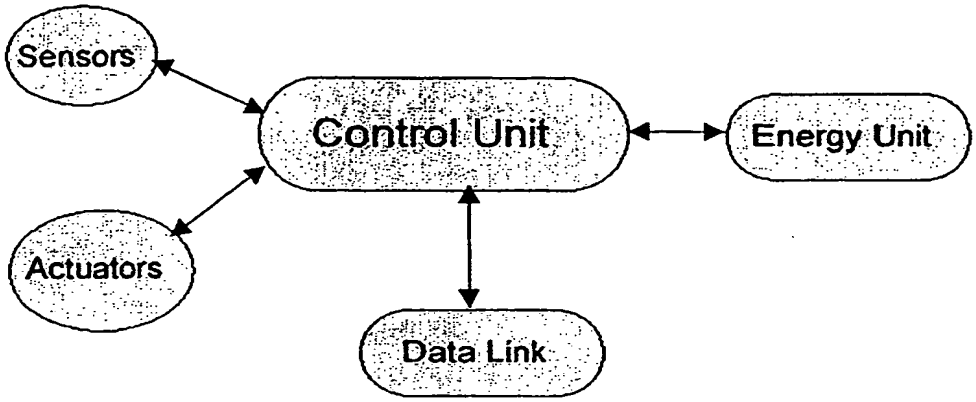
Figure 1



A METHOD OF PRODUCING TISSUE STRUCTURES

The invention provides a method for preparing a scaffold suitable for biomedical use, either *in vivo* or *in vitro*, and to a novel scaffold *per se*. In addition, the invention provides a method for depositing cells (including micro-organisms and cell organelles) onto a surface to promote their growth. The invention provides a method and structure incorporating one or more implants. The invention, in particular, relates to the field of tissue engineering including tissue repair and cell transplantation.

Donor scarcity following successes in the medical field of whole organ transplantation together with the risk of organ rejection and increasing patient demand and expectation, has led researches to consider new ways of repairing and replacing damaged or failed organs and tissues in the human body. The field of cell transplantation using 2 and 3-dimensional matrices, known as scaffolds, to design and grow organs and tissues for transplantation is now in its infancy. The design of the scaffolds so that they are biocompatible, biodegradable, promote cell adhesion and growth has proven to be a major limitation on the advancement of this field.

There have been reports in the literature of cells

harvested *in vitro* and then placed onto biodegradable artificial polymers in culture to form a rudimentary polymer-cell scaffold which can be implanted into a host where vascularisation, growth and function of the cells can occur (*J. Vacanti et al.*, Journal of Paediatric Surgery, Vol. 23, No. 1 (January), 1988, pp. 3-9). Designs for the polymer scaffold include small wafers of biodegradable polyanhydride and fibre networks of biodegradable polymers.

The design of scaffolds conventionally is based on 2-dimensional disk-like structures (*C.A. Vacanti et al.*, Plastic and Reconstructive Surgery, November 1991, pp. 753-759) or extrusion or gelling techniques (U.S. Patent No. 5,290,494), and has not allowed for satisfactory cell growth and differentiation. U.S. Patent No. 5,696,175 describes a method of preparing a 3-dimensionsal scaffold for use as a template for cell transplantation which involves:

1) selection of polymers to form fibres and to form a matrix around the polymeric fibres,

2) selection of a solvent or temperature required to produce a solution of the matrix polymer which does not liquify the fibre polymer,

3) solidification of the matrix polymer around and between the polymeric fibres,

4) heat treatment of the fibre-matrix to immobilise the

fibres where they overlap or cross link, and

5) removal of the matrix polymer to leave immobilised cross linked fibres.

There is a need, however, to provide 2 and 3-dimensional scaffolds which have good processability, are biocompatible, biodegradable as required by their end application, have a high surface area to volume ratio, are mechanically strong and which are able to be processed into preselected complex shapes, have preselected areas permeability and porosity and are able to incorporate bioactive agents and sensing/monitoring devices as required. To date the scaffolds available in the prior art have not provided the necessary degree of flexibility in their design so that the structure of the scaffold is matched to the end application.

An ideal scaffold for a biomedical application which would include tissue and cell transplantation and repair, has a high surface area to volume ratio which produces a significant amount of cell to surface interaction. The surface of the scaffold must have a suitable surface chemistry in order to allow cell attachment and proliferation or indeed, to prevent cell attachment, for example, to prevent adhesions which are undesirable in nerve regeneration. The average pore size of the scaffold and its macroscopic dimensions are important factors

associated with the rate of cell proliferation and nutrient supply. Pore size should be sufficient to allow cells to grow in multiple layers if desired. Optimum pore size is variable depending upon the intended application of the scaffold. Furthermore, the scaffold degeneration rate must be predictable and controlled in order to match the rate of tissue regeneration when the scaffold is implanted *in vivo*. Control of the scaffold properties can be achieved by varying the composition of the particles and components used to make the scaffold, varying the interconnecting pore structure, varying the macroscopic structure, the pore size distribution and varying the pore geometry.

The invention provides a method of producing a scaffold suitable for biomedical application which comprises electrostatically depositing particles of one or more biocompatible material onto a substrate wherein said particles interlink.

The particles deposited by the method of the present invention are solid or partially solid particles of biocompatible material. The particles are capable of being crosslinked or bonded together to form an interlinked structure. The particles of the biocompatible material are prepared by methods conventionally available in the prior art including drying or partial drying

techniques such as freeze drying, critical point drying, air drying prior to milling, and final particle sizing using conventional sizing sieves.

Desiccated cells, micro-organisms, for example, yeast and cell organelles, for example, mitochondria used according to an embodiment of the invention also fall within the scope of "particles", as used herein.

The particles used in the method of the present invention are not size dependent, however, a preferred range of sizes include particles from 10 to 500 microns in diameter, more preferably from 10 to 200 microns in diameter. Particles may either be regular or irregular in shape and include short fibre lengths.

Preferably, the method of the invention employs particles of two or more different sizes in order to obtain a scaffold with regions of preselected differences in permeability.

The particles of the invention are formed from any biocompatible material or mixtures thereof. Additionally, the particles may be mixed with one or more bioactive agent. The bioactive agent may be deposited in accordance with the method of the invention, or it may be added to a scaffold made according to the invention by a conventional

technique, such as, as a liquid, by conventional coating techniques etc.

Biocompatible material is a substance that ideally is not harmful or toxic to living tissue. In reality the word is used to mean a substance which only exhibits minimal/acceptable levels of harm and/or toxicity to living tissue.

Biocompatible polymers include polymers which are either biologically derived or synthetic polymers. Synthetic biocompatible polymers are widely available in the prior art and source references include U.S. Patent No. 5,399,665 and U.S. Patent No. 5,010,161 which are incorporated herein by reference. Biocompatible agents may be biodegradable or non-biodegradable. Many applications require the scaffold to be biodegradable in order to prevent risk of infection and undesirable biological responses.

Suitable biocompatible material includes biologically derived material including bioresorbable material, such as, collagen, elastin, fibronectin, fibrinogen, laminin, fibrin, thrombin, glycosaminoglycans (for example, hyaluronic acid), chitosans and other biosynthetic saccharides, polyhydroxyalkanoates, alginate and other seaweed derived polysaccharides, hydroxyapatite (calcium

hydroxide). Examples of suitable synthetic polymers include polyesters, polyamides, polyurethanes, polyorthoesters, polyacrylonitriles, polyphosphazenes and polyanhydrides and blends and copolymers thereof. Preferred examples include polyglycolic acid, polylactic acid and copolymers of glycolic and lactic acid. Such synthetic biocompatible polymers are preferably biodegradable, for example, the polymers are resorbable when implanted *in vivo* either by enzymatic action or hydrolysis. Although this is preferred, certain non-biodegradable polymers may be suitable for certain applications, for example, ethylenevinyl acetate, polymers of acrylic acid and methacrylic acid.

The biocompatible material according to the present invention may also be selected from synthetic or modified/recombinant forms or fragments of biologically derived resorbable substrates such as hyaluronan (derivatives of hyaluronanic acid), polypeptides, integrin binding RGD (-arginine-glycine-aspartic acid-) sequence, petrapeptide sequences, poly(amino acids) e.g. poly(L-lysine), "pseudo" (poly amino acids) (i.e. amino acids linked other than by amide bonds, elastin amino acid repeat sequence VPGVG (-valine-proline-glycine-valine-glycine-) etc.

Bioactive agents according to the present invention

include any agent which is compatible with and has a bioactive affect on the patient. The bioactive affect may be biological, chemical or biochemical.

Bioactive agent: any substance which exhibits a biological effect. The bioactive affect may be biological, chemical or biochemical. This includes, drugs, antibodies (natural or monoclonal/recombinant or antibody fragments), chemicals (e.g. CuSO4), hormones, growth factors, angiogenesis factors, apoptosis controlling factors, anti-inflammatory agents, cytokines, gene therapy (including liposomal, viral, plasmid and "naked" DNA delivery), vaccines, vitamins, enzymes, recombinant molecules (e.g. RGD amino acid repeat sequence), nutrients, biological building blocks e.g. lipid, protein, polysaccharide)

Please note that all the above can be in a natural or a recombinant version or fragments. Bioactive agents can be used singularly or in combination.

Bioactive agents which provide a chemical affect include agents such as agents which promote protein crosslinking and, therefore, increase the strength of the scaffold (copper ions, for example, copper sulphate and zinc ions) leechable agents which may be leeched out after formation of the scaffold to increase porosity (for example, alginate beads) ascorbic acid, beta-tricalcium phosphate

which is an osteo-conductive material to stimulate new bone growth, etc. The electrostatic deposition method according to the present invention allows the bioactive agents to be selectively distributed within the scaffold to provide a constant rate or pulse controlled release of the bioactive agent and/or to form a concentration gradient within the scaffold. The bioactive agent may be an agent such as ascorbic acid which enhances tissue proliferation.

Other additives which may be incorporated into the scaffolds include substances which enhance the structure and/or the performance of the scaffold, for example, the mechanical strength, handling properties, porosity, degradation or dissolution rate, cell attachment efficiency and surface finish.

When two or more different types of particles of the biocompatible materials and/or are bioactive agents are used to prepare the scaffold of the invention, the particles may be deposited together or separately either from different nozzles or attachments in the same apparatus or via different apparatus. Alternatively, the bioactive agents may be encapsulated, embedded or trapped within the scaffold. These agents may be applied to the scaffold by deposition according to the method of the invention or by conventional means, for example,

conventional coating techniques. Encapsulation may be as a liposome which is prepared prior to its application onto the surface of the scaffold to encapsulate the bioactive agent.

The electrostatic deposition of particles according to the present invention allows for the properties of the scaffold to be controlled and indeed, to be varied across the scaffold structure. Electrostatic deposition allows a mixture of particle types to be applied to a substrate. For example, it is known that natural polymers such as collagen have disadvantages if used solely in that they can provoke adverse tissue and immune responses. However, collagen is known to have good mechanical strength and therefore, is a useful biocompatible material for forming a scaffold. Similarly, synthetic polymers such as polylactic acid, polyglycolic acid and copolymers thereof are known to be good biocompatible and biodegradable agents that are easy to process. However, when used on their own they do not allow flexibility to vary the properties of the scaffold depending on its end application. According to the present invention, therefore, it is possible to vary the composition of particles electrostatically deposited onto a surface.

In addition, the properties of the particles *per se* can be altered in order to affect the mechanical properties of

the scaffold. Particles may be used which vary in mechanical and physical properties in order to produce scaffolds which have preselected regions having different properties. For example, the properties of the powder particles which may be varied include particle size, electrical receptivity, Tribo and Corona charging characteristics [Two commonly used methods of charging powder are "Corona" charging and "Tribo" charging. A Corona charging type of spray gun charges the powder by generating free electrons (negative polarity). These are emitted from the point of a charging needle and ionise the surrounding air. The free ions then attach themselves to the powder particles giving them a negative charge. It is the discharge current (microamps) and not the discharge voltage that charges the powder. Tribo charging is achieved by means of a frictional process inside the spray gun. The powder flow path through the gun is made from insulating material e.g. Teflon. The gun design ensures the maximum amount of friction is applied to the powder particles which forces them to swirl through the spray gun and rub against the inner surfaces. Thus there occurs a number of charge exchange processes between the particles and the gun walls.], hygroscopiscity, fluidity and shape distribution. These properties play a significant role on the performance of powder coating such as transfer efficiency, film thickness, adhesion and overall appearance. Therefore, altering any of these variables in

a controlled manner can result in the production of a scaffold with desired characteristics for example, variations in particle size during production result in a scaffold with controlled variable permeability or porosity.

The method of the invention comprises electrostatically depositing the particles onto a surface. The method of depositing the particles is based on electrostatic powder coating technologies. The methods include, but are not limited to, applying charged powder particles to a neutral substrate, applying neutral powder particles to a charged substrate, applying charged powder particles to a charged substrate, and creating a fluidised bed where a gas is forced through a porous membrane/grid around a substrate and applying a voltage thereto.

We have found that electrostatic deposition provides a more efficient method for producing a scaffold in terms of speed and area over which the particles may be deposited. Electrostatic deposition allows improved coverage of particles over a surface and wrap around of the deposited particles. The process of the invention has the benefits of simplicity and improved flexibility.

According to an embodiment of the invention, the target surface, such as the mould/form, existing scaffold,

medical device, bioreactor etc., may have preselected regions of differing, electrical potentials, or areas with no charge. The effect of this selective charge distribution is to cause the deposited particles to stick to specific regions of the substrate, building layers of differing thickness.

Possible methods of electrostatic deposition include the following:

(a) **Applying charged powder particles to a neutral or charged substrate such as a mould/form.**

Using this technique particles are charged using friction (Tribo charged) and/or by electrical discharge (Corona discharge) prior to deposition. They are then directed towards (using a gas/liquid propellant, or gravity feed system) or put in close proximity (i.e. using a fluidised bed) to the substrate, such as, the mould/form. The resulting attractive electrostatic force that exists between each particle and the substrate increases in strength as they travel closer to the substrate. This attractive force has two benefits, firstly it increases the number of particles reaching the substrate, for example, the mould/form, secondly it enables particles to travel to regions using non line of sight trajectories (wrap around), and finally it helps to anchor the particles to the surface at the point of impact.

b) **Applying uncharged particles to a charged substrate, such as, the mould/form**

Here uncharged particles are directed towards (using a gas/liquid propellant, or gravity feed system) or put in close proximity (i.e. using a fluidised bed) to a charged substrate, such as, a mould/form. The charged substrate creates an electric field. The uncharged particles within the field undergo electrostatic induction and become bipolar due to charge redistribution. The particles are then attracted to the substrate and deposited on its surface as described in (a). The substrate can be charged either by an electrostatic process (such as removing or adding electrons using friction) or by applying a DC or AC signal from an external power pack.

The method of the invention additionally comprises apply cells to the scaffold and promoting their growth, by employing conventional cell culture techniques.

The invention further provides a method of depositing living structures e.g. cells, micro-organisms (e.g. yeast), cell organelles (e.g. mitochondria) onto a surface to promote their growth and proliferation or to immobilise and secure in position. The method involves depositing desiccated cells/micro-organisms/cell organelles onto a substrate, for example, an existing scaffold, which may

have been prepared in accordance with the invention, a mould or form, or directly onto a wound or tissue in the patient. The cells are linked either by applying a linking agent or promoter, utilising receptors on cells to from an inbuilt linked natural linkage. Receptors on cells which may be used to promote their linkage either to each other or to the scaffold or substrate on which they are deposited include natural receptors, such as, integrin receptors on cell surfaces which interact with the RGD amino acid sequence on fibronectin, or receptors formed by recombinant technology. Cells/micro-organisms/cell organelles may also be trapped in position by subsequent/additional layers of scaffold material. The cells/micro-organisms/cell organelles are rehydrated to promote their growth. In the case of desiccated mammalian cells e.g. fibroblasts, keratinoctes rehydration is achieved by adding Dulbecco's modified Eagle's medium (Gibco BRL, Rockville USA) supplemented with 10% bovine calf serum (Gibco BRL, Rockville USA) using conventional tissue culture techniques at 37 degrees C with 10% CO2 in air in order to grow, culture and mature the cells on the scaffold. Initially great care must be taken to prevent the newly deposited cells being washed off the scaffold by the culture medium.

Cells may be dried by firstly protecting the cells from the extreme nature of the dehydration treatment. One

possible method is to add a protective sugar, such as trehalose, to the cells, for example, as a surface coating or by recombinant DNA procedures to engineered cells to produce enzymes to make the protective sugar. For example, Ning Guo *et al* report the preparation of genetically engineered human primary fibroblasts using a recombinant adenovirus vector to express the otsA and otsB genes of *E. Coli* which encode trehalose biosynthetic enzymes. Transformed cells produce increased amounts of trehalose with increasing multiplicity of infection by the vector. Human primary fibroblasts expressing trehalose were found to be viable after being stored in a dried state (Ning Guo *et al*, Nature Biotechnology, vol. 18, February 2000 p.p. 168-171).

Similarly, Ali Eruglu *et al* report that the introduction of low concentrations of intracellular trehalose can greatly improve the survival of mammalian cells during cryopreservation. The authors of this paper (Nature Biotechnology, vol. 18, February 2000 p.p. 163-167) have shown that a genetically engineered mutant of *Staphylococcus Aureus* using α-haemolysin to create pores in the cellular membrane makes it possible to load trehalose into the cells. Low concentrations (0.2M) of trehalose permit long-term post-thaw survival of more than 80% of 3T3fibroblasts and 70% of human keratinocytes.

It is possible, therefore, to use these techniques in the invention to prepare cells for deposition by protecting the cells from the extreme effects of freeze drying or dehydration and thus prepare desiccated cells (the teachings of these two references are incorporated, herein, by reference).

The deposition of desiccated cells according to the invention additionally includes uncharged coating techniques as well as electrostatic coating techniques. Using such techniques, uncharged cells are directed towards an uncharged substrate such as a mould/form or scaffold using a gas or liquid propellant or gravity feed system. Modifications may apply, for example, in order to enhance adhesion of the cells to the substrate, the surface of the substrate may be wet or a layer of liquid or sticky substance may be applied to the substrate in order for the applied cells to adhere and become anchored into position, for example, by using liquid fibronectin.

According to the method of the invention the particles or cells are linked to each other to form the scaffold structure. The linkage of particles may be due to a number of different means, for example, as a result of crosslinking particles or chemically bonding the particles. Means for achieving the linkage of particles

may be chemical or mechanical, for example physically trapping the particles by another substance, or by irradiation, for example, by UV/gamma irradiation. Other methods of linking particles within the scope of the invention include drying the particles, thermally linking the particles, or optically encouraging the particles to link. Chemical cross linking agents may be applied to the scaffold to cause linkage of the particles. The cross linking agents may be in the form of a powder, liquid or gas which may be applied by spraying using conventional methods, electrostatic deposition, dipping the scaffolding into a bonding solution, condensing a crosslinking agent or by direct application. The crosslinking agents may be deposited with the particles onto the substrate, for example, at the time of applying the particles the cross linking agent is in sustained release form, or the crosslinking agent is deposited via a different nozzle in the apparatus or by a separate apparatus. For example a "double-barrelled" arrangement, this is where the two nozzles are strapped together. This apparatus can consist of a Wagner PEM-CGI electrostatic spray gun physically attached in parallel to a Badger (single action external mix spray gun) model B250-2. The output from each gun can be independently controlled or linked.

Alternatively, the particles according to the invention may be preconditioned, for example, exposed to cross

linking agents such as a nebulised acid or exposed to bioactive compounds, for example, nebulised copper sulphate solution, prior to deposition.

Specific examples of chemical cross linking agents according to the invention include acids, for example, hydrochloric acid, calcium chloride, copper, copper sulphate or mixtures thereof. Copper ions increase, for example, the cross linking of fibronectin and fibrinogen and when used in cell culture optimise extra cellular matrix production by increasing smooth muscle collagen synthesis. Other examples of cross linking agents include hexamethylene diisocyanate, gaseous formaldehyde, or combinations of the two. These two crosslinking agents are particularly suitable for crosslinking type I collagen, but may be used generally within the scope of the invention.

The surface onto which the particles are deposited according to the present invention may be selected from a solid object such as a mould or form, an existing scaffold either made according to the present invention or conventionally available in the prior art, a medical device or a bioreactor. The substrate sets the structure of the scaffold and will be selected according to the intended use of the scaffold.

A form or mould may be selected from any hollow, porous or solid structure composed of an inert material, for example, plastics material which optionally can be withdrawn, for example, a balloon offered as the substrate and then deflated once the scaffold is formed. The mould may form an integral part of the scaffold or may serve as a prop in the formation of the scaffold only. The mould may be of a non-inert material, for example, a frozen acid or wax which can be melted out once the scaffold is formed. The form or mould may be a 2-dimensional structure such as a flat sheet or a 3-dimensional structure such as a tube or more complex structure depending on the end use of the scaffold. The form or mould may be a single or multipart structure. For example, if the mould is in two or more parts the resulting scaffold would then need to be assembled by bonding together the separately formed parts in order to complete the structure.

The invention also relates to the production of a scaffold which incorporates a medical device. The medical device will necessarily form an integral part of the scaffold *per se*. The scaffold applied to the medical device may have a number of functions, for example, to increase biological acceptability or performance or to protect the medical device from adverse affect. Examples include coating man-made vascular prosthesis and heart valves, for example,

left ventricular assist devices in order to promote rapid cell attachment, for example, endothelial cells and therefore, reducing thrombus formation within the device. Other uses include adding a layer of cartilage to man-made joints, for example, knee replacements. The present invention relates to the medical devices formed by the method of the invention *per se*.

The substrate according to the method of the present invention may also be a bioreactor. The scaffold is coated onto the bioreactor in order to encourage cell attachment, micro-organism attachment, cell organelle, antibody attachment, enzyme attachment etc. A bioreactor is an apparatus in which a biological reaction or process is carried out, this includes laboratory bench type experiments, pilot plant and industrial applications. Examples include immobilised enzyme and immobilised whole cell catalysts, techniques commonly used in the fermentation industry e.g. production of L-lysine amino acids using immobilised Microbacterium ammoniaphilum, and the production of Penicillin G from immobilised Penicillin chrysogenum.

Therefore, the invention covers composite structures, particularly preferred are structures comprising a scaffold not prepared according to the invention, for example, by extrusion and a scaffold prepared according to

the invention.

Examples of substrates particularly suitable for deposition of desiccated cells include substrates composed of man made materials formed from metals e.g. replacement hip joint/knee joint/fracture fixation plate, synthetic materials e.g. permanent suture material nylon polyamine), prolene (polypropylene), resorbable suture material e.g. dexon (PGA), vycril (polyglactin) and PDS (polydioxanone), Gortex (registered trademark) e.g. used in vascular bypass grafting, Dacron (registered trademark) e.g. used in vascular bypass grafting, etc. The cell coating of the prosthesis would have many benefits including allowing the production of extracellular matrix e.g. chondrocytes applied to metal joint surfaces to produce cartilage to improve joint function or allow early cell coverage e.g. stop the production or thrombi/emboli in synthetic vascular grafts.

Examples of substrates particularly suitable for deposition of desiccated cells include substrates composed of non man made materials include decellularised organs e.g. a decellularised porcine heart valve which can have human cells applied and matured (i.e. extracellular matrix produced) prior to surgical implantation.

The invention also provides as a substrate a scaffold

prepared according to the prior art or in accordance with the present invention.

A scaffold produced by the method of the invention may be a 2- or 3-dimensional structure of interlinked particles of one or more biocompatible material in which the scaffold has distinct regions of preselected permeability. The biocompatible, optionally together with a bioactive agent may be any of the agents available in the prior art, and exemplified above.

The scaffold may be a 2-dimensional structure such as a membrane or mat or a 3-dimensional structure such as a tube or matrix. The scaffold may comprise the substrate on to which the particles were deposited, or alternatively, the substrate may have become disassociated from the scaffold. The scaffold may additionally be coated with one or more additives by conventional techniques. The overall structure of a scaffold is not limited and numerous possibilities exist due to the flexibility of the method of the invention, for example, it may be a single layer of crosslinked material, multiple layers, laminates of crosslinked layers (ie a paper mache effect), a flat sheet of crosslinked material rolled concentrically to produce a rod or tube, assembled from separately prepared parts, a composite structure etc.

Preferably, the scaffolds according to the present invention comprises a mixture of different biocompatible and/or bioactive agents.

Since scaffolds according to the present invention comprise particles deposited onto a substrate and then crosslinked, it is possible to control and alter the properties of the scaffold either across its entire surface, or in distinct regions. For example, it is possible to have control over the degree of crosslinkage between the particles. Increasing the amount of crosslinking in collagen, for example, leads to a reduction in the degradation rate, a decreased capacity for collagen to absorb water, a decreased solubility and an increase in tensile strength in the resultant fibres. A similar result is observed with synthetic hyaluronic acid derivatives.

In addition, the permeability and/or the porosity of the scaffold can be varied, either across its entire surface, or in distinct regions. Scaffolds for some applications may ideally be highly porous structures with the majority of pores connected to one another. Therefore, the scaffolds of the present invention may have controlled variable porosity in all 3-dimensions throughout the structure. This can be achieved, for example, by varying the particle size, varying the particle composition,

varying the particles shape, varying the hygroscopicity of the particles, or a combination of these techniques.

The ability to vary the composition of the scaffold improves the properties of the scaffold and ensures far greater degree of control and design of the scaffold for its desired function. Controlling the composition allows control over degradation rates, strength and flexibility.

As the scaffolds of the present invention are formed by depositing particles onto a surface and then linking the particles, it is possible to include in the deposition method reinforcable material analogous to steel reinforcing rods in concrete. For example, short hydroxyapatite fibres may be deposited or added to desired areas in a scaffold in order to produce reinforced regions of high strength.

Porosity can be affected by controlling the formation of voids in the scaffold structure. The formation of voids may be encouraged by forming gas bubbles within the deposited particles forming the scaffold, for example, carbon dioxide bubbles produced by exposing the wet scaffold to carbon dioxide gas under pressure so that the gas dissolves into the wet scaffold material. Pressure is then reduced and bubbles form in the scaffold. Other methods of forming voids and controlling their formation

and size, include adding carbonic acid to the scaffold when just formed and reducing the temperature. The scaffold may then have regions of isolated voids, interconnecting voids, surface cavities or any combination.

Pores may also be introduced into the scaffold by spraying frozen droplet particles, for example, by modifying the technique described by McGregor J. et al (U.S. Patent No. 5,869,080), in order to allow it to be used with spray technology i.e. spraying either using electrostatic charged or not particles of a substance that has a low sublimation point such that once the sublimation temperature is reached the solid becomes gas and exits the scaffold thus leaving a void/pore in its place. The substance can be mixed with the linking agent e.g. hexane (volatile water-immiscible hydrocarbon) mixed with hydrochloric acid(linking agent) prior to spraying or sprayed separately during the scaffold manufacturing stage. This stage must be performed at a temperature below the sublimation point for the pore forming substance.

The internal surface of the scaffold takes up the surface appearance of the substrate onto which the particles are deposited, for example, the internal surface may be a flat glass shiny surface (after drying) if the substrate is a

flat glass surface onto which the cells are deposited. The external surface of the scaffold may then be texture varied as appropriate, for example, the surface may be grooved, pitted, punctured (i.e. a channel connecting the inner and outer surfaces of the scaffold) or indented.

The invention also encompasses an embodiment which provides a scaffold which has embedded into its surface either internally or on an external surface, an implant either for use *in* vitro or *in* vivo.

The scaffold comprising the implant may be a scaffold according to the invention or a scaffold prepared according to the prior art, or a composite structure comprising a scaffold prepared according to the invention and a scaffold according to the prior art.

The scaffold may comprise cells of one or more types which have been deposited onto its surface, and wherein growth and differentiation of the cells has been promoted. One or more implants may be incorporated into the cells. Alternatively, an implant may be attached to the external surface of the scaffold to become embedded on the surface of the next layer of cells. An embodiment of the invention relates to cells grown on a scaffold comprising one or more implants wherein the scaffold has degraded.

An implant may also be incorporated into the embodiment of the invention which relates to the deposition of desiccated cells. The implant may be embedded within the cells or attached to the surface of the cells.

Implants can be either biodegradable or non biodegradable. Implants can be biocompatible or non biocompatible. If they are non biocompatible they will require partial/total encasing in a biocompatible material in order to prevent harmful contact between the host tissues and the device but still allowing the device to operate, e.g. encasement of a non biocompatible glucose sensor in a biocompatible material with pore sizes that allow through substances the size of glucose molecules in order to interact with the device but not so large as to allow immune cells/antibodies to come into direct contact with the device. Suitable materials are well known and include plastics, metals such as stainless steel, titanium etc.

An implant is any man-made structure that is implanted into or onto a scaffold during its manufacture or after its manufacture or in the case of the embodiment of the present invention relating to deposition of cells, an implant is embedded into the actual cells/extra cellular matrix directly.

This is a man made device comprising of one or more

components that interact with each another using signals (e.g. electrical, optical, acoustic). The signals can contain instructions and/or data, and/or energising power. The implant has the capability to process, filter, store, receive, or transmit signals depending on its function.

Examples include temperature sensors, for example, temperature sensors embedded into a PGA scaffold prepared in accordance with the present invention, or prepared in accordance with conventional methods, such as by extrusion or melt moulding, a displacement sensor, for example, one encapsulated into a scaffold produced by the electrostatic powder coating according to the present invention, a metal wire added to a scaffold after its production, or during its production, and held in place by a tissue friendly adhesive, for example, fibronectin, a pressure sensor, for example, one embedded between the layers of a blood vessel manufactured using the technique described by L'Heureux (A completely biological tissue-engineered human blood vessel; N. L'Heureux, S. Paquet, R. Labbe, L. Germain, F. A. Auger, FASEB Journal, 12, 47- 56 (1998)), such as by concentric winding. This implant is eventually or partially encapsulated by cells, extra cellular matrix.

The implant may be removed during any stage of the use of the scaffold, for example, either *in vitro* or *in vivo*, or it may be retained permanently after implantation into the

host organism. Methods of removal of the implant include active removal, for example, physical removal, or passive removal, for example, allowing the implant to degrade or biologically break down.

Other implants include sensors for determining biochemical affects in the body, for example, sensors for determining sugar concentration. The invention also relates to scaffolds which include a combination of sensors. Some specific examples include a temperature sensor. Other examples include sensors which may be used to monitor cell growth and differentiation when the scaffold is implanted *in vivo*. Other examples of mechanical sensors includes transmitters which would be embedded into the scaffold which may then be controlled externally when the scaffold is implanted *in vivo*, for example, to stimulate muscle control.

Implants added to scaffold either according to the invention or available in the prior art, or added to tissue engineering structures prepared according to the invention or in accordance with conventional techniques, may be made to function during the period of cell attachment to the scaffold, either *in vivo* or *in vitro*, and/or during subsequent growth stages. Alternatively, the implant may be made to function after implantation has occurred, either immediately after or at the time of

implantation, or at sometime afterwards, depending on the use of the scaffold or tissue engineering implant, for example, up to many tens of years after implantation. The implant may be functional continuously during these periods or intermittently. If the implant is used intermittently, then it may be activated by an external signal, for example, a radio wave, following a pre-set pattern built into the implant or transmitted to it, for example, using radio waves or memory devices. Alternatively, the implant may respond to environmental conditions, for example, it may be triggered by specific chemical agents, specific biochemical agents, by chemical reaction, pressure, heat, radiation etc. A single implant may function over a combination of these periods, or alternatively, a scaffold or tissue engineering structure according to the invention may include a number of different implants.

Some particular uses and beneficial affects of implants according to the invention are:

- The implants act as an interface between traditional biomedical devices e.g. pace maker, left ventricular assist device and tissue engineered structures allowing them to interact. The tissue engineered structure could for example be a heart comprising cardiac muscle but no bundle of His/natural

conducting pathway requiring pressure sensing in the chambers of the heart, conduction of signal, signal processing, conduction of signal to a pacemaker and electrically pacing (closed loop feed back).

- The implant could act as a connector between a biomedical device and a paralysed organ e.g. implants could be imbedded into biologically friendly structures which are attached to the muscle. These implants could sense tension/displacement in the muscle and process the signal (+/- in combination with simultaneous data from other muscles) in order to trigger other implants to stimulate the muscle to contract. Similarly implants could be used to allow diabetics with peripheral neuropathy (loss of sensation) to be alerted to possible tissue damage.

- An implant can be used to create an electric and/or magnetic field around (and inside) the scaffold/cell structure. The fields generated being used to assist in cell attachment (e.g. growth patterns/alignment, growth rates). The fields can be applied continuously, intermittently, and/or modulated to change their properties (e.g. shape, amplitude, frequency, phase).

- Living conditions of the cells can be monitored directly and appropriate action taken to enhance these conditions. Examples; 1) temperature measurement, and then reduce or heat the cell growth region accordingly to an optimum level 2) pH measurement and then adjust the environment accordingly.

- Regions of the host or transplanted organ/part organ can be stimulated to assist natural functions or restore natural functions. E.g. the implant providing electrical impulses to trigger or assist in the triggering of muscle activation (pace maker for the cardiac muscle, limb movement in the case of skeletal muscle).

- Sensation e.g. pressure, proprioception, vibration sound, light in regions of the host or transplanted organ/part organ can be detected using an appropriate sensor.

- They can assist in organ care/management during periods where the cell structure is outside the host, either being modified, repaired, stored or transported. The care/management helping with the survival and regeneration of the cells and the

production of extracellular matrix.

- Quality control during production e.g. sensing minimum burst pressure during blood vessel manufacture.

- Performance of implanted tissue and the host (e.g. patient) can be monitored over time. The data generated being used to improve the quality, performance and life span of the tissue for the host.

- The transplanted cells can be tracked by giving the implant a unique identification, which can be accessed remotely.

- The implant may contain a history of the cell structure, thereby assisting in diagnosis and treatment of the host, operating, for example, as an aircraft Black Box, giving a history of the cells performance and the conditions under which their performance was undesirable e.g. when they were subject to dehydration, skeletal muscle going into a spasm or if the tissue engineered heart had an arrhythmia (irregular cardiac rhythm).

There are a number of possible implant configurations

envisaged by the present invention. A list is given below, and a particular example is illustrated in Figure 1, which provides an example of a system containing all the possible components available, and their interconnection.

Although the components within an implant may be integrated into a single region it is possible to identify five distinct components as illustrated in Figure 1. The central component in the system is the control unit which is where signals can be stored, processed, transmitted to other devices and/or generated. The control unit requires energy to operate and so it is connected to an energy unit, which is responsible for energy storage and/or energy conversion. The data link enables the control unit to communicate with other external devices. Sensors provide input signals about the environmental conditions to the control unit. They may also be supplied with energising, stimulating, and/or data signals from the control unit to enable them to function. Finally the last section is actuators, this section is responsible for changing the environmental conditions and/or stiumulating a reaction in the host and/or implanted region.

Implants according to the invention may fall into the following categories:

(1) **Measurement systems:** comprising of one or more sensors (e.g.: electrical, optical, opto-electronic, acoustic, opto-acoustic, biological) measuring one or more measurands (e.g. temperature, pH levels, electrical activity, conductivity, pressure, tension/compression, change of displacement, osmolarity, substance concentration e.g. glucose or CO2). The signals from the sensors can be (i) processed, (ii) stored in memory for later transmission, (iii) stored in memory for signal processing, or (iv) instantly transmitted to another implant(s), or remote electronic system (external to the host or cell structure containing the implant). The remote system can then process the signal from the embedded implant and if necessary change the environmental conditions of the cell/scaffold structure.

(2) Control systems: comprising of one or more actuators/transducers (e.g.: micro-pumps for drug release, electrodes for electrical stimulation of muscles or cell alignment). The control signals for the actuators/transducers being stored in memory or transmitted to the implant from another implant or from remote circuitry external to the host or cell structure.

(3) **Measurement and control systems:** This is a combination of (1) and (2). Some or all of the sensor signals providing feedback (either negative or positive)

to help control the operation of some or all of the actuators/transducers (e.g. drug delivery rate too low... increase dose rate, cell temperature too cold ... activate heater, heart rate too low activate the pace maker to increase heart rate). The signal processing can be carried out locally within the implant, or remotely, by communicating with another implant or remote external circuit.

All of the above configurations may require the following components

- **An energy source** The electrical energy required to energise the components within the implant can be supplied by an energy storing device (e.g. battery, capacitor), an energy conversion element (solar cell, induction circuit), a fuel cell, or a radioactive power source (e.g. pace maker)

- **Data link** This allows data to be transmitted to and/or from an implant. The communication may be to another implant, or an external circuit. Data transmission can be achieved using (i) optical signals radiated directly through the scaffold/cells, or guided along optical fibres. (ii) electrical signals along conductors (i.e. hardwired connections) or transmitted as electromagnetic radiation (e.g.:

radio/microwaves) (iii) acoustic waves transmitted through the tissue/cells/scaffold.

It is possible to establish a data link between the implant and a commercial communication network/system using satellite, fibre optic electrical cable or wireless technology (e.g.: telephone, cellular phone, WAP phone, and the World Wide Web) either directly, or using additional external electronic/opto-electronic circuits. The implant can then transmit and receive data to any location around the world.

There are a number of advantages associated with the scaffold or tissue including the implant. The following is not intended to be limiting:

- Condition/function of organ/cells and/or host can be remotely monitored and recorded in databases e.g. based at the tissue engineering manufacturer or immediately acted upon.

- An alarm signal can be transmitted by the implants to alert patient, medical teams or patient's physician.

- Location of patient can be ascertained when alarm signal is sent.

- Function of organ/tissue with implant can be treated/altered by physician anywhere in the world.

- Organs can be tracked during transit.

- Organs can be tracked once implanted in patients.

The following examples provide working examples of the method according to the present invention. The examples are illustrative of the invention and are not intended to be limiting.

Experiments carried out to create finished scaffolds

Scaffold fabrication was broken down into a two-stage process; (i) basic scaffold construction and (ii) end processing. Basic scaffold construction consisted of applying protein particles and crosslinking agent layers to a mould/form to create the desired scaffold shape and scaffold thickness. There were a number of ways of achieving this first stage, four of which are detailed below. The second stage of end processing was common to all experiments after the basic scaffold construction had been achieved.

The experiments described below employed either alginate,

fibronectin/fibrinogen, collagen or a mixture of some or all of these components in either a dry/partially dry form. The alginate was sodium alginate (Sigma product number 30105) produced from Laminaria hyperborea. The fibronectin/fibrinogen was prepared using the cryoprecipitate/PEG precipitation described by Sarah Underwood (PhD thesis "The production of human material for skin replacement" successfully submitted November 1998 to University College London) and the freeze dried using an Edwards Mini Fast 680 Mk 2 freeze drier prior to milling an particle size sorting. The particles were sorted (sieved) into the following ranges (microns); <10, 10-53, 50-75, 75-125 and >125 using sieves from Endecotts (Test Sieves) Ltd., Wimbledon, England. Particles were either used from a particular range or a mixture. The collagen was bovine collagen type I (Sigma product number C3511).

The crosslinking step used in these particular experiments was carried out sequentially at room temperature using 2 different solutions. Crosslinking solution 1 was 0.25M HCL/2% calcium chloride/1 microM CuSO4 and crosslinking solution 2 was 2.0M HCL/2% calcium chloride/1 milliM CuSO4.

Stage 1 Basic Scaffold construction

Example 1: Applying electrically charged protein

particles onto a mould/form

To enable the scaffold to be inspected at any point in its development a glass microscope slide was used as the mould/form. The slide was clamped in a vertical position using an all metal (i.e. electrically conducting) retort stand. The stand was fitted with an earth strap to ground to ensure the stand remained electrically neutral.

(The Examples were carried out at room temperature). Using a Badger airbrush (single action external mix spray gun) Model B250-2 containing crosslinking solution 1, the glass slide was sprayed with tiny droplets until a thin film was produced. Negatively charge powdered protein particles were then sprayed at the slide using the spray gun of a Wagner manual powder coating system 2007 (EPG 2007 control unit, PEM-2C gun and ME-1 airfluid unit with PJ 2020 PRS injector).

The corona discharge spray gun was held approximately 10 cms from the slide and gently waved to and fro, to ensure an even coating was deposited on the wet slide. Once a uniform dry layer of powder had been deposited another fine layer of crosslinking solution 1 was deposited using the airbrush. The amount used being sufficient to wet all of the deposited dry protein particles. Visually the particles looked more translucent once they had been

sufficiently coated in crosslinking solution 1. The next layer of protein was then applied using the powder spray gun followed by crosslinking solution 1, etc. The powder/crosslinking solution 1 cycles were repeated to increase the scaffold thickness. Finally the scaffold was 'end processed' using the stage 2 technique described below.

In this experiment crosslinking solution 1 performed three functions. Firstly it provided an electrical conducting surface over the mould/form and provided a conduction path to the earthed retort stand, thereby ensuring the crosslinking solution 1 mixture film was maintained at zero volts (electrically neutral). Secondly the crosslinking solution 1 helped trap impacting protein particles with its surface tension effects. Thirdly crosslinking solution 1 started the bonding/crosslinking of trapped protein particles to one another.

It should be noted that the Wagner powder spray system was used with in input air pressure of 0.6 bar and an output voltage of 80 kV DC. The Badger airbrush was connected to a commercial pressurised canister (750 ml Badger airbrush propellant BP750).

A variation of this experiment using a 4mm Perspex rod as the mould/form. This was gradually rotated during

building up layers of protein powder and spraying with the cross linking solution 1. The `end processing' was carried out as detailed below and a cylindrical scaffold was produced.

Example 2: Applying electrically neutral protein particles onto a charge mould/form

The mould/form in this experiment took the form of a small rectangular sheet of tin foil 4cm by 3cm held tightly in contact (using Cellotape) with the surface of the collecting sphere of a WBN, Scotland Van de Graaff generator. The potential on the surface of the collecting sphere was estimated to be around 750kV with respect to ground (i.e. zero volts).

The Van de Graaff generator was switched on and given 30 seconds for it to reach its maximum operating voltage. Electrically neutral (i.e. uncharged) protein particles were sprayed at the tin foil target (at a distance of 25 cms) using the powder spray gun and control system described in experiment 1 with the voltage to the gun set to zero volts. The powder from the gun formed a uniform layer on the tin foil target. The Badger airbrush described in experiment 1 was then used to wet the particles as described in experiment 1. Subsequent powder/crosslinking solution 1 layers were used to build

up the scaffold thickness to a desired amount. Finally the scaffold was 'end processed' using the stage 2 technique detailed below.

Example 3: Applying electrically neutral desiccated mammalian cells onto an electrically neutral scaffold coated with a thin layer of liquid fibronectin using a powder spray gun

This experiment was carried using the same experimental set up and technique described in example 1 except that no deflector cone was used. In addition to this the voltage at the powder spray gun electrode (where corona dischage occurs) was held at zero volts by earthing the discharge electrode. The scaffold, a 1x1 cm square of fibronectin/fibrinogen was prepared using this invention. The cells used were desiccated human primary fibroblasts prepared using the method described by Guo and Puhlev (Trehalose expression confers desiccation tolerance on human cells; (2000) Nature Biotechnology vol 18, p168-171) After basic scaffold construction was completed the scaffold was washed in Tris buffer and then water to remove traces of the HCl crosslinking agent. Finally it was gamma irradiated and then under aseptic conditions coated with a thin layer of fibronectin liquid prior to cell deposition. This was achieved using the spray gun of

a Wagner manual powder coating system 2007 (EPG 2007 control unit, PEM-2C gun and ME-1 airfluid unit with PJ 2020 PRS injector) except that no deflector cone was used. In addition to this the voltage at the powder spray gun electrode (where corona discharge occurs) was held at zero volts by earthing the discharge electrode. A thin layer of desiccated cells was deposited. The fibronectin was allowed to dry thus securing the desiccated cells. The cells were rehydrated to promote their growth. This was acheived by adding Dulbecco's modified Eagle's medium (Gibco BRL, Rockville USA) supplemented with 10% bovine calf serum (Gibco BRL, Rockville USA) using conventional tissue culture techniques at 37 degrees C with 10% CO2 in air in order to grow, culture and mature the cells on the scaffold. Great care must be taken when initially adding the culture medium to prevent the newly deposited cells being washed off the scaffold.

Example 4: Applying electrically neutral desiccated mammalian cells onto an electrically neutral scaffold using a gravity feed system

The scaffold, a 1x1 cm square of fibronectin/fibrinogen was prepared using this invention. The cells used were desiccated human primary fibroblasts prepared using the method described by Guo and Puhlev (Trehalose expression

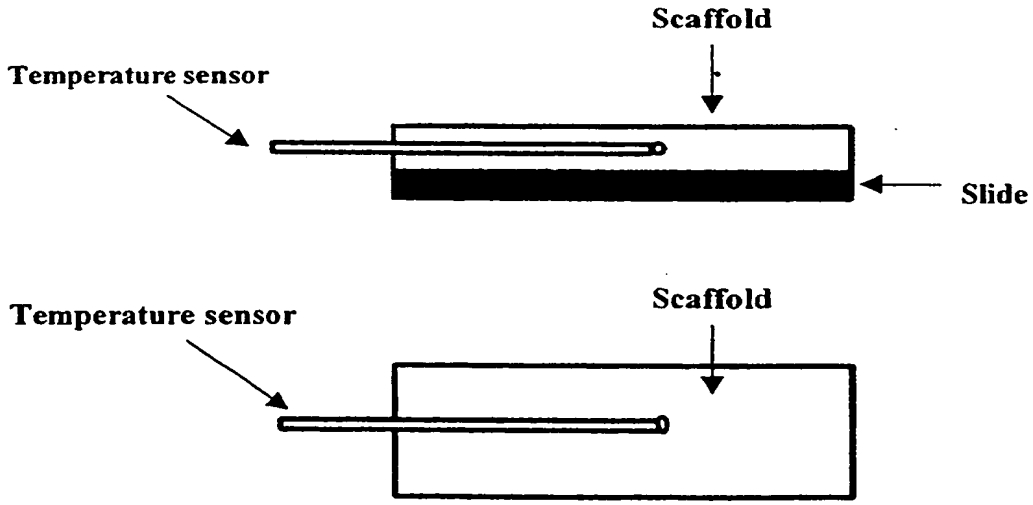
confers desiccation tolerance on human cells; (2000) Nature Biotechnology vol 18, p168-171) After basic scaffold construction was completed the scaffold was washed in Tris buffer and then water to remove traces of the HCl crosslinking agent. Finally it was gamma irradiated and then under aseptic conditions coated with a thin layer of fibronectin liquid prior to cell deposition. The desiccated cells were then sprinkled under aseptic conditions onto the wet fibronectin coated scaffold until a thin layer of cells had been deposited. Sprinkling was achieved using one of two ways. The first was to simply shake by hand desiccated cells from a test tube and the second way was to use a conventional salt cellar filled with desiccated cells. The procedure then followed that of example 3 above.

Example 5

Embedding a temperature sensor into scaffold produced using the electrostatic technique described in example 1.

The form mould was a glass microscope slide. After five layers of fibronectin had been deposited and linked using crosslinking solution 1 a temperature sensor (Type K thermocouple 0.5mm in diameter manufactured using stainless steel supplied by Farnell, order no. 721-8898) was positioned parallel to and in contact with the coating

such that the sensing tip was in the central region. The sensor was 150mmm in length and was held in place using an additional clamp. A further ten layers of fibronectin were deposited and crosslinked. This resulted in the temperature sensor being embedded into the scaffold. The scaffold was end processed as described in stage 2. The thermocouple was connected to a Digitron temperature indicator module supplied by RS, order number 258-186. This implant provides a means to monitor the temperature during cell attachment, growth and maturation.

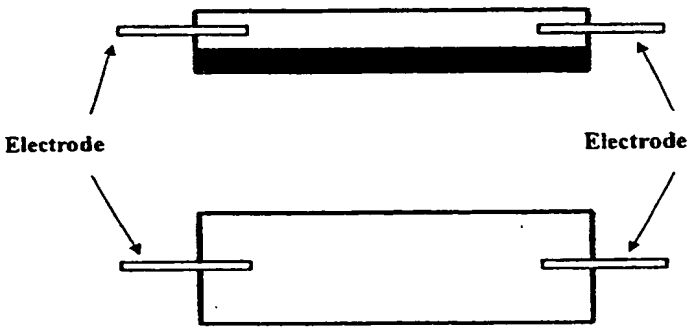


Top diagram: Longitudinal cross-section. Lower diagram: plan view

Example 6

Embedding electrodes into a scaffold to stimulate skeletal muscle cells.

This experiment was identical to example A, except that 0.1mm diameter stainless steel wires 20cms in length were imbedded instead of the temperature probe. The wires were positioned parallel to the surface, at the edge with only 5mm protruding over the over the slide's surface. One wire was placed at either end of the slide as shown in the diagram below. After skeletal muscle cells have been attached, the wires can be used to transmit electrical impulses to the scaffold to stimulate/activate the developing muscle.

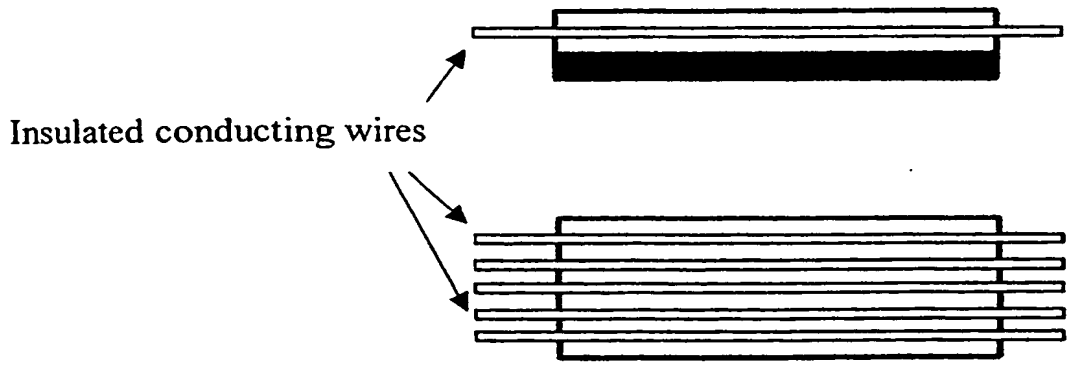


Top diagram: Longitudinal cross-section. Lower diagram: plan view

Example 7

Embedding conductors into a scaffold to enhance cell growth and alignment.

This experiment was identical to example A except that instead of a temperature sensor being used 5 insulated copper wires 30 cms in length (RS 357-918) were laid parallel to each as illustrated in figure 1. After cell attachment current is passed along the wires in order to generate both a magnetic and electric field. The current can be dc ac or pulsed.



Top diagram: Longitudinal cross-section. Lower diagram: plan view

Stage 2: End processing

After the basic scaffold (stage 1) had been achieved and left for a period of 30 minutes in order to air dry, the scaffold was immersed (drenched) in cross linking solution 2 for 60 seconds to ensure further bonding/cross linking of the protein particles. The scaffold was then allowed to air-dry again and then immersed/drenched in buffer solution (200mM Tris) for 60 seconds. The scaffold was then air dried again and then washed in water and then allowed to air dry/partially dry to leave interconnected structure on the mould/form, or removed from the mould/form whilst still slightly damp to give a stand alone scaffold structure. Finally some of the scaffolds were gamma irradiated to increase crosslinking and also to sterilise the structure.

i) The method and scaffolds of the invention are suitable for biomedical applications. Such applications particularly apply to the field of tissue engineering, in particular, in the field of repair, regeneration or replacement of tissues and organs. Tissue engineering allows the use of living donors such as close relatives of the patient or the patient himself, or the cells may be from another source, such as, stem cells, neonatal cells, for example, neonatal foreskin cells and avoids the need

for whole organ transplantation through donation from a deceased donor. Cells may be harvested from a donor, cultured *in vitro* on a 2 or 3-dimensional scaffold according to the invention for a time sufficient for cellular viability to be established or the cells may be deposited in accordance with the method of the present invention, and then transplanted into the patient where vascularisation, growth and differentiation are promoted.

A number of possibilities are encompassed by the invention in the field of tissue engineering. The following are examples thereof:

i) A scaffold is produced in accordance with the invention and cells, but more likely just bioactive agents are applied to the scaffold. The scaffold is then implanted into the host, for example, as a synthetic blood vessel or flat sheet of collagen impregnated, for example, with $CuSO_4$/vitamin C, and used to treat burns etc. Thus, the scaffold is itself functional on implantation. The scaffold may also include one or more implants according to its use.

ii) A scaffold prepared in accordance with the invention may be used to promote growth of damaged tissue within the host. For example, if a nerve is damaged, a scaffold in the form of a tube is implanted to join

up the damaged ends. The scaffold has two functions in that it prevents unwanted cells from filling the gap between the damaged nerve endings and promotes growth of the nerve endings to assist repair. The scaffold may comprise bioactive agents and/or implants as appropriate. Hence the scaffold is acting as a scaffold bandage in traumatic nerve repair. The scaffold may also be used in this way to promote the growth of other tissues, for example, skin over a wound or ulcer, or in the repairs of cartilage or bone.

A scaffold according to the invention may be used to promote the growth and differentiation of tissue or organs for subsequent implantation. For example, blood vessels may be grown on a scaffold by applying cells around the scaffold (endothelial cells on the inside and smooth muscle cells on the outside). The cells are grown for a sufficient period of time to promote their differentiation (e.g. 12 weeks). As the cells grow and form a blood vessel, the scaffold begins to break down either partially or completely. The blood vessel may then be transplanted into the host. An implant, such as one or more sensors, may be incorporated into the blood vessel, for example, between the endothelial cells and smooth muscle cells. Although the scaffold degrades over time, the

implant, such as a sensor, remains as part of the structure. The implant may also be incorporated into a tissue or organ formed by other means, for example, a blood vessel formed by the technique described by L'Heureux (A completely biological tissue-engineered human blood vessel; N. L'Heureux, S. Paquet, R. Labbe, L. Germain, F . A. Auger, FASEB Journal, 12, 47- 56 (1998))

iii) The scaffold may also be used for tissue engineering part of or whole organs, such as, liver, pancreas, bladder, cardiac muscle and cardiac valves.

Other biomedical applications include the following uses. The invention provides a method for depositing desiccated cells onto a surface, such as a scaffold. Alternatively, cells may be attached to scaffolds prepared according to the invention by conventional techniques, such as traditional cell seeding techniques. The desiccated cells deposited according to the present invention are capable of being rehydrated in order to stimulate growth.

Other suitable uses are in the field of gene transduction, for example, delivering virus, modified virus particles, DNA, liposome coated DNA etc., to cells.

Other suitable uses include pharmacological uses, in particular, in the area of cell cultures and tissue

cultures in order to test drugs or other chemicals for toxic, physiological, biological, chemical, biochemical, pharmacological and mechanical properties in pre-clinical studies. Specific areas include studying the ageing process, repair mechanisms, cancer growth and treatments, blood clotting, radiation interaction, for example, the affects of UV radiation on tissue engineered skin, and in gene therapy.

Further uses of the scaffolds according to the invention are in the area of medical device testing, for example, analysis of the affects of man-made substances, for example, metal in prosthetic hip joints on tissue and cells *in vitro* and *vice versa*.

Scaffolds of the present invention may also be analysed to assess the intrinsic properties of scaffold materials, for example, collagen sheets for haemostatic control.

The scaffolds of the present invention may be used as biosensors, for example, by attaching enzymes, DNA and/or proteins to the scaffolds in order to measure a specific variable.

The method of the invention concerned with the deposition of cells may be used for direct *in vivo* application, for example, by spraying particles directly on to a wound and

linking the particles *in situ*.

DNA micro-array technologies e.g. DNA chips i.e. facilitating the attachment of genetic material e.g. DNA, CDNA, RNA to a suitable surface e.g. a glass rod for the purpose of diagnostics/gene discovery/drug discovery/toxicology research.

The examples of biomedical uses for the methods and scaffolds of the invention are not intended to be limiting.

CLAIMS:

1. A method of producing a scaffold suitable for biomedical application which comprises electrostatically depositing particles of one or more biocompatible material onto a substrate wherein said particles interlink.

2. A method according to claim 1 which additionally comprise applying cells, micro-organisms, cell organelles or combinations thereof to the scaffold and promoting their growth.

3. A method according to claim 1 or claim 2 wherein said biocompatible material is selected from biocompatible, biodegradable or non-biodegradable, natural or synthetic polymers or mixtures thereof.

4. A method according to any of claims 1 to 3 wherein said particles are mixed with one or more bioactive agents.

5. A method according to claim 4 wherein said bioactive agent is selected from antibodies (natural or monoclonal/recombinant or antibody fragments), chemicals (e.g. CuSO4), hormones, growth factors, angiogenesis factors, apoptosis controlling factors, anti-inflammatory agents, cytokines, gene therapy agents (including

liposomal, viral, plasmid and "naked" DNA delivery), vaccines, vitamins, enzymes, recombinant molecules (e.g. RGD amino acid repeat sequence), nutrients, biological building blocks e.g. lipid, protein, polysaccharide) or combinations thereof.

Please note that all the above can be in a natural or a recombinant version or fragments.

6. A method according to claim 4, wherein said bioactive agent is selected from leechable agents to increase porosity, strengthening agents, agents to enhance tissue proliferation and agents to promote cell growth (e.g. $CuSO_4$).

7. A method according to any of the preceding claims wherein the particles vary in size to induce varying porosity over the cross section of the scaffold.

8. A method according to any of claims 1 to 6 wherein the particles vary in hygroscopicity to induce varying permeability over the cross section of the scaffold.

9. A method according to any of the preceding claims wherein said substrate is selected from a mould or preform, an existing scaffold, a medical device or bioreactor.

10. A method according to claim 9 wherein said substrate is an existing scaffold resulting in a composite structure.

11. A method according to any of the preceding claims wherein the particles are deposited on to said substrate by a method selected from electrostatic powder coating of said substrate and electrostatic powder suspension coating of said surface.

12. A method according to any of the preceding claims wherein the substrate has preselected regions of differing electrical potential.

13. A method according to any of the preceding claims wherein the particles are interlinked by chemical bonding.

14. A method of promoting cell growth which comprises depositing onto a substrate desiccated cells, wherein the cells interlink, and promoting growth of said cells by rehydration.

15. A method according to claim 14 wherein said substrate is a scaffold.

16. A method according to claim 14 or claim 15 wherein

said cells are protected by a sugar prior to dehydration or freeze drying.

17. A method according to any of the preceding claims wherein one or more implants are attached to said scaffold or to said cells.

18. A method according to claim 17 wherein said one or more implant is embedded within the scaffold or cells, attached to the surface of the scaffold or cells, or a combination thereof.

19. A scaffold suitable for biomedical application which comprises one or more implants.

20. A scaffold according to claim 19 wherein said scaffold is a 2- or 3-dimensional structure of interlinked particles comprising one or more biocompatible materials.

21. A scaffold according to claim 19 or claim 20 wherein said one or more implants are attached to a surface of said scaffold, embedded within the scaffold, embedded within or attached to a layer of cells grown on said scaffold or a combination thereof.

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Application No: GB 0007756.0 **Examiner:** L.V.Thomas
Claims searched: 1-13, 17(part) and 18(part) **Date of search:** 17 August 2000

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Databases searched:

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UK Cl (Ed.R):

Int Cl (Ed.7):

Other: Online: EPODOC, WPI, BIOSIS, MEDLINE, CAS-ONLINE, SCISEARCH, EMBASE

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
A	WO 99/18892 A1	(CAMBRIDGE SCIENTIFIC) see p.2 ll.12-35	1
X	WO 94/13266 A1	(UNIV. OF CALIFORNIA) see p.4 l.8 - p.5 l.18, p.6 l.32 - p.7 l.21 and p.9 ll.1-13	1,3,11
X	GB 1377022	(AVICON, INC.) see p.1 ll.12-69 and p.4 ll.6-22 and 59-79	1,3-5,11
X	GB 1221952	(RANSBURG CORP.) see p.1 ll.16-66 and p.2 ll.53-102	1,3,11

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
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